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SELECTIVE FERTILIZATION AND THE RATE OF POLLEN-TUBE GROWTH.

D. F. JONES,

CONN. AGRIC. EXPER. STATION, NEW HAVEN, CONN.

A difference exists in the ability of pollen from dissimilar plants to accomplish sexual fusion when acting in competition. This has been shown by the writer (1920) with maize (*Zea mays* Linn.) and the tomato (*Lycopersicum esculentum* Mill.). Two other cases of a similar effect have been reported. These will be referred to later. In these species the plant's own pollen is more efficient in completing fertilization than pollen from plants having somewhat different genetical construction, and with maize the superiority of self-fertilization is greater as the germinal differences increase.

The method used to prove that there was an inequality in fertilization with maize was to make a mixture of approximately equal quantities of pollen from two different lots of plants, each of which possessed a dominant seed character. The mixture was applied at the same time to the plants which furnished the pollen, and when the seeds were mature it was possible to separate the seeds resulting from the two kinds of pollen on both types of plants. For example in some of the tests a white, smooth-seeded variety was contrasted with a yellow wrinkled variety. In one case the self-fertilized seeds were white and the cross-fertilized yellow, and in the other the contrast was between wrinkled and smooth seeds. The four classes of seeds in the two types of inflorescence were quite distinct and easily separated and counted. The numbers when arranged in the form of a proportion showed that there was a marked selective action in favor of self-fertilization.

The greatest difference in fertilizing efficiency was found when a small seeded variety of popcorn of the *Zea mays everta* type having very corneous and pointed seeds was used. The selective action was so pronounced that it seemed worth while to repeat the tests with this type on a more extensive scale. At the same time, the pollinations were made and the data recorded in such a way as to give an indication as to whether or not the result was due to a differential rate of pollen tube growth.

The seeds of maize are arranged regularly on a central spike. Each ovule has a separate pistil and these form a mass of fine filaments which extend beyond the enclosing leaf sheaths. See Fig. 1. Just before the pollinations were made the pistils were cut off evenly at a short distance beyond the tip of the spike, and the pollen mixture was applied to the cut ends of the filaments. The distance that the pollen tubes had to travel to reach the ovules differed considerably in the case of the seeds produced in the tip of the spike as compared with those at the base. The mature pistillate inflorescences of the material worked with ranged from ten to twenty centimeters in length. At the time fertilization took place they were considerably shorter than this. It is estimated that the pollen tubes travelled through a distance which varied from about five to fifteen centimeters. If the plant's own pollen tubes grow faster than the foreign tubes, we would expect fewer cross-fertilized seeds at the base of the spike than at the tip.

Five different mixtures of pollen were made and applied to about ten plants of each of the two contrasted types. For the white, smooth-seeded type a first generation hybrid of two inbred strains of a variety of sharply pointed popcorn known as Squirrel Tooth was used, and for the yellow, wrinkled type another first generation hybrid of two inbred strains from a variety of sweet-corn known as Golden Bantam. Hybrid plants were employed because of their size and vigor, making it possible to secure a large number of seeds from a single application of pollen. The plants of each type were characteristically uniform, and were practically identical in genetical constitution. They were producing segregating gametes but these were presumably alike in respect to their cytoplasmic covering. In any case the gametic differences were no greater than are present in the original varieties. The

same two types of plants were used in all five pollen mixtures. The mature inflorescences resulting from the application of mixed

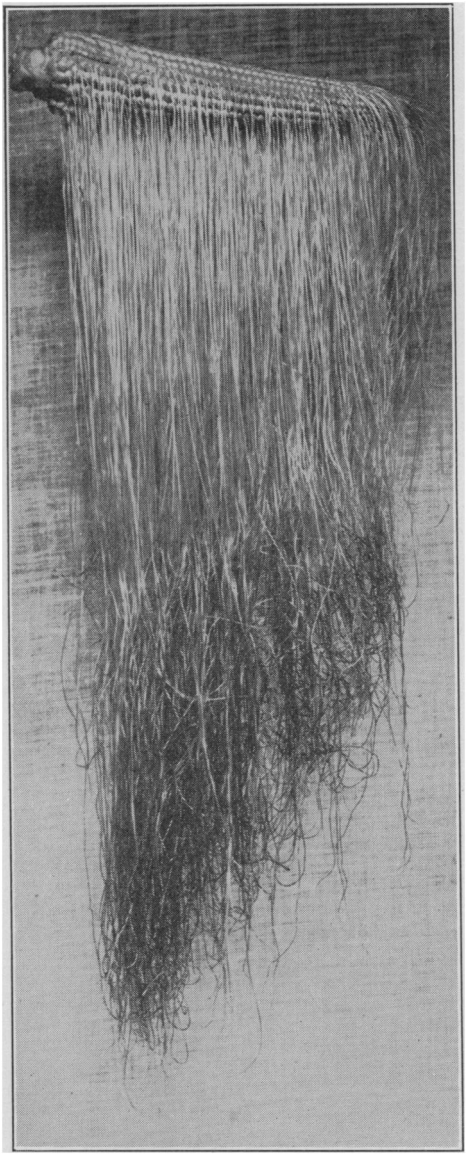

FIG. 1. The pistillate inflorescence of maize at the time of pollination with leaf sheaths removed. (About one half natural size.)

pollen were divided into two approximately equal halves and the

seeds from the top half and bottom half were counted separately. The data from the entire spikes are brought together and given in Table I. to present the amount of selective action shown. The

TABLE I.

THE AMOUNT OF SELECTIVE ACTION SHOWN BY MAIZE IN FIVE POLLEN MIXTURES.

Pollen Mixture No.	Number of Seeds.				Total Number of Seeds.	Deviation from Perfect Proportion in Per Cent.
	A × A.	A × B.	B × A.	B × B.		
1.....	811	11	381	2,006	3,209	41.35
2.....	4,222	27	466	1,404	6,119	37.22
3.....	1,568	2	319	224	2,113	20.56
4.....	1,930	29	73	309	2,341	39.71
5.....	4,084	6	963	290	5,343	11.50

figures from all the plants used with each mixture are combined and are arranged in four classes in the form of a proportion. If there were no differences in fertilizing ability the proportion should be a perfect one within the limits of random sampling. The white, smooth-seeded plants are designated *A* in the tables, and the plants with yellow wrinkled seeds when uncrossed are listed under the heading of *B*. The number of seeds resulting from *A* pollen and from *B* pollen on *A* plants should be in the same ratio as the numbers from the same two kinds of pollen on *B* plants irrespective of the amount or viability of each kind of pollen in the mixture. As in the numerous cases previously reported such is clearly not the result obtained, as there are much fewer cross-fertilized seeds than would be expected if fertilization took place at random. The selective action is in favor of the homogeneous union as was found before and the differences are marked. The last column gives the deviation from the closest calculated perfect proportion based upon the per cent. of cross-fertilized and self-fertilized seeds on each type of plant. The maximum deviation possible is 50 which would indicate complete functioning of each kind of pollen on its own flowers, but not at all on the others, or vice versa as the case might be. The deviations actually range from 12 to 41 in favor of self-fertilization, in each case, and show a very high degree of selective action, amounting in some cases to almost total inability of the extrane-

ous pollen to bring about fertilization when acting in competition. Fig. 2 shows a pair of mature pistillate inflorescences resulting

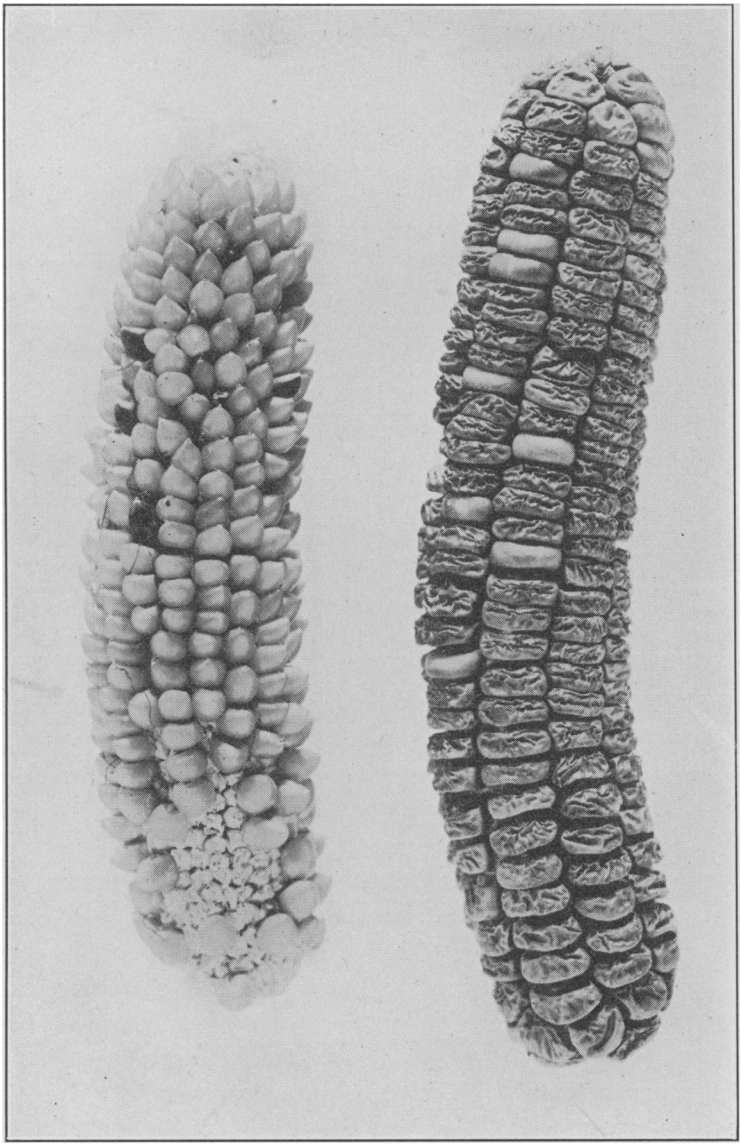

FIG. 2. Mature inflorescences resulting from the application of a mixture of pollen from the two kinds of plants which produced them. (About three fourths natural size taken on orthochromatic plate with Wratten C blue color filter.)

from pollen mixture number 1. The cross-fertilized seeds stand out unmistakably on both plants, but are few in number.

The data which show that this selective action is due in part, at least, to differences in rate of pollen-tube growth, are arranged in Table II. The total number of seeds in the top and bottom

TABLE II.

THE DISTRIBUTION OF CROSSED SEEDS IN MAIZE INFLORESCENCES RESULTING FROM MIXED POLLINATIONS.

Pollen Mixture No.	Plant Type.	Number of Seeds.		Total Number of Seeds.	No. of Crossed Seeds per Hundred.		Excess of Crossed Seeds per Hundred in Top Half.
		Top Half.	Bottom Half.		Top Half.	Bottom Half.	
1.....	*A*	452	370	822	1.99	.54	1.45
1.....	*B*	1,219	1,168	2,387	17.23	14.64	2.59
2.....	*A*	2,126	2,123	4,249	1.22	.05	1.17
2.....	*B*	979	891	1,870	26.66	23.01	3.65
3.....	*A*	811	759	1,570	.25	.00	.25
3.....	*B*	298	245	543	60.07	57.14	2.93
4.....	*A*	1,023	936	1,959	2.54	.32	2.22
4.....	*B*	186	196	382	24.73	13.78	10.95
5.....	*A*	2,075	2,015	4,090	.10	.20	−.10
5.....	*B*	612	641	1,253	78.43	75.35	3.08

halves of the inflorescences, as divided arbitrarily before shelling, are roughly equal. The number of cross-fertilized seeds per hundred of all seeds is more in those seeds which resulted from the shorter lengths of pollen tubes. Only one exception is noted, and here there were only six crossed seeds in a total of over four thousand. In all the others, positive differences are shown, but these, however, are not large, so that one cannot be sure whether or not the inequality in fertilization is due entirely to differences in the rate of pollen-tube growth. Corroborative evidence has been furnished by Miller (1919), who has observed that many pollen tubes may start to grow down the style of maize, but in about 100 examinations only one tube was seen to reach the ovary cavity in every case.

A similar selective action favoring the plant's own pollen has been found with cotton. Balls (1919) put an equal quantity of pollen of two distinct cultivated types of this plant (*Gossypium*)

on the stigmas of each. Only ten hybrids were obtained from 330 seeds resulting from the mixed pollen. Likewise Heribert-Nilsson (1920) found that the pollen tubes of *Œnothera gigas* grow slower in the styles of *O. Lamarckiana* than do the latter's own pollen tubes. These results were obtained by cutting off the styles close to the ovary at different times after pollination and noting the shortest time after pollination necessary for seeds to set.

A somewhat different kind of selective fertilization has been demonstrated by Correns (1920) with the diœcious plant *Melandrium*. The staminate plants of the species worked with are heterogamous, and the pistillate plants homogamous, corresponding to the sex conditions in animals of the *Drosophila* type. The pistillate-determining pollen tubes apparently grow faster than those which result in staminate plants. This was demonstrated in the following way. The mature seed-pods were divided into upper and lower halves and the seeds of each grown separately. The seeds from the upper portion resulting from the shorter lengths of pollen tubes gave 68 per cent. pistillate plants, while the seeds from the lower portion gave 56 per cent. of the same type. It should be noted that the conditions are reversed in the case of *Melandrium* and *Zea*. In the former the pollen tubes enter the ovary at a common point and are free to fertilize the first ovules they reach. The tubes which grow fastest therefore fertilize the ovules in the upper part of the ovary, leaving the slower-growing tubes to pass on down to the lower part. In *Zea* each ovule has a separate style so that, the longer the distance to traverse is, the less chance will the slower-growing tubes have of reaching the goal first.

Correns also found that when pollen was applied in large excess as compared to a moderate application, the pistillate plants resulting were always in greater proportion. But even when a deficient amount of pollen was applied there was still a small excess of pistillate plants showing that some other selective factor than differences in rate of pollen-tube growth was operating in addition.

Heribert-Nilsson also obtained aberrant ratios from self-fertilized and back-crossed heterozygous red-nerved plants of *Œno-*

thera Lamarckiana. He interprets the result as due to a selective action between gametes carrying different factors. In this case as well as in the preceding experiments with *Melandrium* two kinds of gametes were produced by the same individual and presumably they were alike in respect to their cytoplasmic covering, differing only within the nucleus. In the case of *Zea, Lycopersicum, Gossypium,* and *Œnothera Lamarckiana* pollinated by *gigas,* the gametes came from different individuals of unlike germinal construction and therefore differed both in nuclear content and enveloping cytoplasm. The two manifestations of selective fertilization may be unrelated. On the other hand it is not unlikely that they may have something in common.

The discrimination which works against the bringing together of individuals of unlike germplasm has been demonstrated by representatives of four distinct orders in the two main classes of angiosperms. In its effect it is comparable to the assortative mating of animals from the lowest to the highest. The predilection for the bringing together of like with like is more evident as the germinal differences increase. There is thus exhibited a tendency which when carried far enough may erect an impassable physiological barrier between different groups that were once compatible. It is an indication that sterility between species is the result of the accumulation of genetic differences, however these differences may arise.

LITERATURE CITED.

Balls, W. L.

'19 The Cotton Plant in Egypt. London.

Correns, C.

'20 Eine geglückte verschiebung des geschlechtverhältnisses. Natur u. Technick, Vol. 2, pp. 65–71.

Heribert-Nilsson, N.

'20 Zuwachsgeschwindigkeit der Pollenschlaüche und gestörte Mendelzahlen bei Oenothera Lamarckiana. Hereditas, Vol. I, pp. 41–67.

Jones, D. F.

'20 Selective Fertilization in Pollen Mixtures. BIOLOGICAL BULLETIN, Vol. 38, pp. 251–289.

Miller, E. C.

'19 Development of the Pistillate Spikelet and Fertilization in *Zea Mays* L. Journal Agricultural Research, Vol. 18, pp. 255–266.